



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Roderick Scott  
Serial No. : 10/058,825  
Filed : January 30, 2002  
Title : MODIFIED PLANTS

Art Unit : 1638  
Examiner : Stuart Baum

Commissioner for Patents  
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF STEVEN E. JACOBSEN

I, Steven E. Jacobsen, declare as follows:

1. I am a citizen of the United States and presently reside at 29087 Saddlebrook Drive, Agoura Hills, CA 91301.
2. I received a B.S. degree in 1987 from California Polytechnic State University in San Luis Obispo, California, majoring in Crop Science. I received a Ph.D. degree in 1993 from the department of Plant Biology at the University of Minnesota. I was employed as a post-doctoral researcher in the laboratory of Dr. Elliot Meyerowitz at the California Institute of Technology from 1993 to 1998.
3. I am presently a Professor of Molecular, Cell and Developmental Biology at the University of California, Los Angeles (UCLA) and have been so employed since 2003. I was employed as Assistant Professor and Associate Professor of Molecular, Cell and Developmental Biology at UCLA from 1998 to 2003. I am currently an Investigator of the Howard Hughes Medical Institute and have been so employed since 2005.

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4. I was named a Fellow of the American Association for the Advancement of Science in 2004. I also received the Searle Scholar and Beckman Young Investigator Awards in 2000.

5. I am an author or co-author on a number of publications in refereed scientific journals, and an author or co-author on several review articles. A copy of my bibliography is attached.

6. Major research interests in my laboratory are the genetics of DNA methylation patterning in *Arabidopsis* and genome wide analysis of DNA methylation. I have been involved in research in these subject areas since 1997.

7. I am familiar with the literature regarding the genetics and biochemistry of DNA methylation in plants.

8. I have been engaged by Ceres, Inc. as a consultant with respect to prosecution of U.S. Patent Application No. 10/058,825 ("the '825 application"). I understand Ceres is a licensee of the '825 application.

9. I have read the '825 application, the claims pending as of January 27, 2006 in the '825 application, and the Examiner's Office Action mailed January 27, 2006 for the '825 application.

10. It is my opinion that, in 1999, a researcher in the area of plant molecular biology would not have interpreted a partial *Arabidopsis* or *Zea mays* DNA methyltransferase 1 Met1 sequence as used in the pending claims to comprise only two nucleotides from the *Arabidopsis* or *Zea mays* DNA methyltransferase 1 Met1 sequences.

11. One of ordinary skill, after reading the '825 application, would have realized that the subject matter of the application relates, *inter alia*, to the use of *Arabidopsis* or *Zea mays* Met1 sequences for downregulation. One of ordinary skill would have known that sequences having

only 2 nucleotides of sequence identity are too short to stably hybridize to a complementary sequence such that downregulation can occur. One of ordinary skill would have known this from, for example, the Cannon et al. reference, which states that they “have found that a 41-base pairing homology was sufficient to give up to a 100% inhibition of GUS expression.” Cannon et al. *Plant Mol. Biol.* 15:39-47 at page 46. The Cannon reference also states at page 39 that “[a]ntisense RNA has a complementary sequence to mRNA and inhibits gene translation by a mechanism as yet unknown but presumed to involve duplex formation.” The Gutterson reference states that sense suppression was observed “at sizes down to 225 bp; however, the proportion of plants with a high level of suppression decreased with decreasing fragment length, most notably below 550 bp.” Gutterson, (1995) *HortScience* 30:964-966 at page 965. Therefore, one of ordinary skill would have recognized that two consecutive nucleotides is not a partial *Arabidopsis* or *Zea mays* DNA methyltransferase 1 *MET1* sequence because a dinucleotide is not of a sufficient length to permit downregulation.

12. I have read the Jacobsen 2000 *Current Biology* reference (Jacobsen et al., 2000, *Current Biology* 10:179-186). The subject matter of the 2000 *Current Biology* reference concerned, *inter alia*, the observation that the *AGAMOUS* gene was hypermethylated in an *Arabidopsis* line expressing a Met1 antisense construct. These lines were previously described as having up to a 90% decrease in overall DNA methylation. Finnegan et al., (1996) *Proc. Natl. Acad. Sci. U.S.A.* 93:8449. An earlier publication, the Jacobsen 1997 reference, showed that the *SUPERMAN* gene was hypermethylated in the same *Arabidopsis* line expressing a Met1 antisense construct, and these experiments also confirmed the simultaneous overall hypomethylation in this line. (Jacobsen et al., *Science* 1997 277:1100-1103).

13. I am the first author of the Jacobsen 2000 reference and the Jacobsen 1997 reference.

14. The observation of hypermethylation of the *SUPERMAN* gene or the *AGAMOUS* gene in *Arabidopsis* Met1 antisense construct-containing lines does not change the fact that these lines

had a significant reduction in the degree of overall DNA methylation. The data about the *SUPERMAN* and *AGAMOUS* genes in the Jacobsen 1997 and 2000 references have no bearing on whether one of ordinary skill would have expected a decrease in the degree of overall DNA methylation upon downregulation of Met1 expression.

15. It is my understanding that the Examiner interpreted the language in claims 20 and 62 regarding a construct comprising a promoter and another sequence to mean that the sequence is located in a position not necessarily next to the promoter and that the promoter would not necessarily affect the transcription of the sequence. Based on my review of the '825 application, one of ordinary skill would consider such an interpretation of claims 20 and 62 to be unreasonable. One of ordinary skill would interpret these claims to mean that a promoter that targets expression to female germ line cells drives transcription of the indicated Met1 sequence, based on statements in the '825 application such as those at page 30, lines 15-19 and Examples 3 and 4.

16. It is my opinion that the techniques required to screen and identify Met1 downregulation construct-containing plants that have a decrease in DNA methylation would have been routine for one of ordinary skill, because the techniques involved would have been typical of those carried out by one of ordinary skill. Such techniques include constructing DNA clones containing partial and full-length *Arabidopsis* or *Zea mays* DNA methyltransferase 1 Met1 sequences, constructing plant transformation vectors, transforming plants, and screening for overall DNA methylation status.

17. I have read the Fourgoux-Nicol reference (Fourgoux-Nicol et al., 1999, Plant Molecular Biology 40:857-872).

18. The Fourgoux-Nicol reference involved the cloning of genes involved in male gametophyte development. Fourgoux-Nicol at page 857, Abstract. The reference reports that the

authors succeeded in isolating 13 cDNAs whose expression was strictly confined to the male gametophyte and was high in the microspore. Fourgoux-Nicol at page 862, right-hand column. Thus, the reference shows that hybridization techniques were used successfully to isolate clones.

19. The authors of the Fourgoux-Nicol reference focused their analysis on one of the thirteen cDNA clones whose expression was strictly confined to the male gametophyte and was high in the microspore. The selected clone was designated M3 and had a length of 497 bp. Fourgoux-Nicol at page 863, left-hand column. M3 was used in a second round of screening by stringent hybridization to isolate a second cDNA clone, designated M3.21. M3.21 has a length of 674 bp. Fourgoux-Nicol at page 863, left-hand column. M3 and M3.21 were sequenced and found to have non-identical sequences, including a 99 base pair insertion in M3 that was not present in M3.21, and several single nucleotide polymorphisms between the two. Fourgoux-Nicol at page 863, right-hand column, and page 862 Figure 2. Further analysis, including Southern hybridization, indicated that the M3 and M3.21 cDNAs were derived from two homologous genes. Fourgoux-Nicol at page 864, left-hand column. Thus, the reference shows that 100% sequence identity is not required in order to successfully isolate related sequences by nucleic acid hybridization.

20. The fact that nucleic acids that do not have 100% DNA sequence identity, such as M3 and M3.21, can hybridize to each other would indicate to one of ordinary skill that hybridization would likely occur between a partial or full length *Arabidopsis* or *Zea mays* DNA methyltransferase 1 Met1 sequence and an endogenous DNA methyltransferase 1 Met1 target even when there is less than 100% sequence identity.

21. I have read the following references: Hibino T., et al. (1995) Biosci. Biotech. Biochem 59:929-931; Bolitho, K. M., et al. (1997) Plant Science 122: 91-99; Elkind, Y., et al. (1990) Proc Natl Acad Sci U S A 87(22): 9057-61; and Salehuzzaman, S. N., et al. (1993) Plant Mol Biol 23(5): 947-62.

22. Hibino et al. report that introduction of an *Aralia cordata* cinnamyl alcohol dehydrogenase (CAD) antisense construct into tobacco resulted in an approximately 20-55% reduction in CAD activity. See page 929 and Figure 1 of Hibino. Bolitho et al. report that introduction of an apple antisense ACC-oxidase reduced the level of RNA and the activity of the corresponding gene in tomato. See Figures 3 and 4 of Bolitho. Salehuzzaman et al. report that introduction of a cassava granule bound starch synthase antisense gene suppressed levels of the corresponding protein in potato. See Figure 10 in Salehuzzaman. Elkind et al. report that introduction of a bean phenylalanine ammonia-lyase (PAL) sense sequence into tobacco resulted in reduced levels of PAL activity and reduced accumulation of endogenous PAL transcripts. See Elkind at page 9059-9060. Each of these references shows that a sequence introduced into a heterologous plant species successfully downregulated an endogenous heterologous gene, either as evidenced by the reduction of the heterologous RNA, the reduction of the protein in the heterologous plant, or the downregulation of the desired trait or activity, even when the sequence identity was less than 100 %. The downregulation was mediated via sense suppression in some cases, and via antisense suppression in other cases.

23. It is my opinion that one of ordinary skill would have expected that in general heterologous partial or full length sequences can be used to downregulate endogenous genes based on, *inter alia*, the successful results reported in the references of paragraph 22.

24. I have read the Emery reference (Emery et al., 2003, Current Biology 13:1768-1774).

25. It is my opinion that one of ordinary skill, after reviewing the Emery reference, would not conclude that use of antisense techniques in plant molecular biology requires a 100% sequence match between an introduced sequence and its target. While the Emery reference reports that mismatches introduced within microRNA target sites can abolish miRNA function, such a result does not mean that sequences with imperfect homology would necessarily be ineffective for

downregulation. The references mentioned in paragraph 22 above show that sequences with less than 100% sequence identity to an endogenous gene have been used successfully to downregulate genes in plants. For example, apple and tomato ACC-oxidase cDNAs have 74% sequence identity. Bolitho, et al. (1997) Plant Science 122: 91-99 at page 96, left-hand column. An apple ACC-oxidase antisense construct successfully downregulated the endogenous ACC-oxidase in tomato. Bolitho et al. Figures 3 and 4. Based on references such as the Bolitho reference, one of ordinary skill would have concluded that antisense sequences with less than 100% sequence identity can be used to downregulate a heterologous endogenous gene.

26. I have read the Gutterson reference (Gutterson (1995) HortScience 30:964-966).

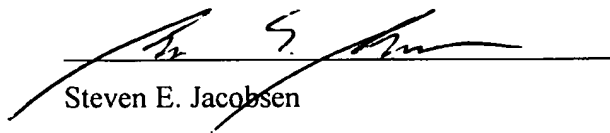
27. It is my opinion that one of ordinary skill, after reviewing the Gutterson reference, would not conclude that use of sense suppression techniques in plant molecular biology requires a 100% sequence match between an introduced sequence and its target. While the Gutterson reference reports that a chrysanthemum chalcone synthase sense sequence did not suppress a petunia chalcone synthase, such a result does not mean that sequences with imperfect homology would necessarily be ineffective for downregulation. The references mentioned in paragraph 22 above show that sequences with less than 100% sequence identity to an endogenous gene have been used successfully to downregulate genes in plants. For example, bean and tobacco phenylalanine ammonia-lyase (PAL) DNAs have 71% sequence identity. Elkind, et al. (1990) Proc Natl Acad Sci U S A 87: 9057-61 at page 9057. Introduction of the bean PAL sense sequence into tobacco resulted in reduced levels of PAL activity and reduced accumulation of endogenous PAL transcripts. Elkind et al. at pages 9059-9060. Based on references such as the Elkind reference, one of ordinary skill would have concluded that sense suppression sequences with less than 100% sequence identity can be used to downregulate a heterologous endogenous gene.

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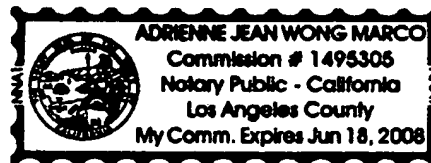
Attorney Docket No.: 11696-067001

28. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity and/or enforceability of the instant patent application or any patent issuing thereon.

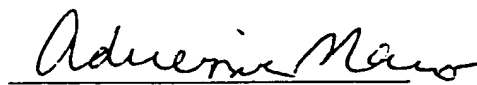
Dated: 7/26/06

  
Steven E. Jacobsen

STATE OF California )  
 ) ss.  
COUNTY OF Los Angeles )



Before me this 26 day of July, 2006, personally appeared Steve Jacobsen known to me to be the person whose name is subscribed to the foregoing Declaration, and acknowledged that he executed the same as his free act and deed for the purposes therein contained.

  
Notary Public



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